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Caloric Restriction and L-Carnitine Administration Improves Insulin Sensitivity in Patients With Impaired Glucose Metabolism

Alessio Molfino, MD; Antonia Cascino, MD; Caterina Conte, MD; Cesarina Ramaccini; Filippo Rossi Fanelli, MD; and Alessandro Laviano, MD

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Background: Reduced circulating and tissue carnitine levels, possibly leading to impaired mitochondrial function, have been postulated to be involved in the pathogenesis of insulin resistance. However, whether L-carnitine administration may improve insulin sensitivity in patients with impaired fasting glucose (IFG) or type 2 diabetes mellitus (DM-2) is still controversial. The aim of the study was to explore the role of L-carnitine supplementation in influencing insulin sensitivity. *Methods:* A randomized controlled study involving adult outpatients was designed. Adult patients referred to the outpatient clinic and within 10 days of the diagnosis of IFG or DM-2 were consecutively enrolled. Exclusion criteria were concomitant antidiabetic therapy and modifications of lifestyle during the previous 4 weeks. Patients were randomly assigned to receive a hypocaloric diet for 10 days (group C; n = 8) or the same dietetic regimen in addition to oral L-carnitine (2 g twice daily) supplementation (group LC; n = 8). Oral glucose tolerance test (OGTT), fasting plasma insulin levels, and homeostasis model assessment of insulin resistance (HOMA-IR) were assessed at the beginning and end of the study. Data were statistically analyzed using the Student *t* test for paired and unpaired data. *Results:* OGTT at 2 hours improved in both groups. Only in the L-carnitine–supplemented group did plasma insulin levels and HOMA-IR significantly decrease when compared to baseline values. *Conclusions:* Considering the role of caloric restriction in increasing the intestinal uptake of carnitine, the results suggest that oral L-carnitine administration, when associated with a hypocaloric feeding regimen, improves insulin resistance and may represent an adjunctive treatment for IFG and DM-2. (*JPEN J Parenter Enteral Nutr.* 2010;34:295-299)

Keywords: insulin resistance; diabetes mellitus, type 2; carnitine; diet

In elderly patients, an age-associated decline in insulin sensitivity has been demonstrated, which might be related at least in part to impaired mitochondrial function.[1] Supporting evidence shows that aging is related to the carnitine content of peripheral tissues and reduced aerobic enzyme activity.[2] Interestingly, and similar to what is observed in elderly people, the rate of glucose oxidation and plasma carnitine concentrations are lower in patients with impaired fasting glucose (IFG) and type 2 diabetes mellitus (DM-2) than in controls.[3] Also, plasma carnitine levels are reduced in patients with diabetes complications.[4] These data suggest that insulin resistance occurring either in the elderly or in younger patients with IFG or DM-2 may have a common pathogenic mechanism involving reduced circulating and tissue carnitine levels.

The clinical relevance of carnitine deficiency and impaired fatty acid oxidation in mediating insulin resistance in patients has yet to be ascertained. Nevertheless, it could be hypothesized that enhancing mitochondrial fatty acid oxidation may yield improved insulin sensitivity. L-carnitine could represent a suitable tool to influence mitochondrial fatty acid oxidation because it is involved in energy metabolism by carrying acyl groups into mitochondria and transporting acetate from mitochondria to the cytosol.[5] Also, L-carnitine is critical in glucose metabolism because it reduces the acyl-CoA/CoA ratio in mitochondria, which in turn increases the activity of pyruvate dehydrogenase and facilitates glucose disposal.[5]

Preliminary reports suggest a role for L-carnitine in influencing insulin resistance. In animals, L-carnitine supplementation has been shown to improve insulin-stimulated glucose disposal and to ameliorate systemic carbohydrate oxidation.[6] In humans, data are scanty and controversial. Positive results have been obtained, suggesting that

From the Department of Clinical Medicine, Sapienza University, Rome, Italy.

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Address correspondence to: Alessandro Laviano, MD, Department of Clinical Medicine, Sapienza University, viale dell'Università 37, 00185 Rome, Italy; e-mail: alessandro.laviano@uniroma1.it.

L-carnitine administration may represent a simple and effective adjunctive treatment in insulin-resistant patients. Derosa et al[7] showed that L-carnitine administration enhances whole-body glucose utilization and lowers circulating lipids. In healthy individuals, an intravenous (IV) bolus of L-carnitine increases glucose disposal and oxidation.[8] Similar effects have been obtained in DM-2 patients receiving an IV infusion of L-carnitine.[9] Furthermore, oral L-carnitine administration in DM-2 patients results in lower fasting glucose levels, although it is unclear whether these effects were due to improved insulin sensitivity.[10] Conversely, it recently has been reported that 4-week oral L-carnitine administration in DM-2 patients fails to modify insulin sensitivity or the lipid profile.[11]

To further explore the role of L-carnitine supplementation in influencing insulin sensitivity and to assess the clinical settings most likely to yield beneficial metabolic effects from the intervention, we designed a randomized controlled study involving adult outpatients with IFG or DM-2 and supplemented their diets with L-carnitine for 10 days.

Patients and Methods

The study protocol was designed according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee at our institution. Adult outpatients within 10 days of diagnosis of IFG or DM-2 were consecutively enrolled in the study. Patients were diagnosed with diabetes or IFG based on the criteria indicated by the American Diabetes Association and by the World Health Organization.[12] Patients on antidiabetic therapy or those who indicated changing their lifestyle during the previous 4 weeks were not considered for the study.

Modification of the diet, particularly of macronutrient selection, is among the first steps in the treatment of patients with impaired glucose metabolism. Considering that enrolled patients were diabetic or had IFG, we believed it was unethical to maintain the usual diet during the study period, having also in mind that antidiabetic medications could not be prescribed. Therefore, to minimize the effects of changes in dietary habits and macronutrient selection, after informed consent, patients were randomly assigned to receive a hypocaloric diet (1,200 kcal/d for women and 1,400 kcal/d for men; 55% carbohydrates, 25% lipids, 20% proteins) for 10 days (group C) or the same dietetic regimen in addition to 4 g/d (2 g twice daily) of oral L-carnitine (group LC). The dosage was chosen according to the existing literature[11] and reflects the availability in Italy of vials containing 2 g of carnitine.

Patients were instructed not to change their lifestyle during the study period. Oral glucose tolerance test (OGTT; values at fasting and 2 hours after glucose load, mg/dL), fasting plasma insulin levels (μU/mL), and homeostasis model assessment of insulin resistance (HOMA-IR) were assessed at the beginning and at the end of the study. HOMA-IR was calculated according to the validated formula HOMA-IR = fasting plasma insulin (μU/mL) × fasting serum glucose (mg/dL)/405.

Plasma insulin and serum glucose levels were measured by the automated chemistry analyzer Olympus AU400 (Olympus Italia, Segrate-Milano, Italy).

Data were statistically analyzed using the Student *t* test for paired and unpaired data (SPSS for Windows, version 16.0; SPSS Inc, Chicago, IL). A value of $P < .05$ was considered statistically significant. Data are presented as mean ± standard deviation (SD).

Table 1. Patient Characteristics at Baseline

	Group C (n = 8)	Group LC (n = 8)
Age, y	64.2 ± 14.5	69.1 ± 12.6
BMI	25.8 ± 6.8	28.6 ± 6.8
DM-2:IFG	3:5	6:2
Fasting glucose, mg/dL	98.4 ± 22.2	110.7 ± 12.0
Plasma insulin, μU/mL	4.5 ± 2.1	7.0 ± 2.6
OGTT 2 hours, mg/dL	193.2 ± 64.1	232.6 ± 64.7

Data are presented as mean ± standard deviation (P = NS). BMI, body mass index; DM-2, type 2 diabetes mellitus; IFG, impaired fasting glucose; OGTT, oral glucose tolerance test; group C, group control; group LC, group L-carnitine.

Results

During the enrollment period, 16 patients matched the inclusion criteria and agreed to participate. Eight patients (7 men, 1 woman) were randomly assigned to group C, and 8 patients (5 men, 3 women) were assigned to group LC. Patient characteristics at baseline are summarized in Table 1. At the beginning of the study, both groups were comparable for all the variables studied, including HOMA-IR.

No adverse reactions were observed in patients receiving L-carnitine. At the end of the study period, no significant differences were observed in fasting plasma glucose levels between the 2 groups, and body weight similarly decreased in C and LC patients (–2.9% ± 1.5% vs –2.7% ± 1.5%, respectively; P = NS). At the end of the study period, glucose metabolism improved, as shown by the significant reduction of OGTT at 2 hours in group C (193.2 ± 64.1 vs 128 ± 53.29; $P = .04$; Figure 1A) and group LC (232.6 ± 64.7 vs 146.2 ± 59; $P = .01$; Figure 1B). A more pronounced reduction of OGTT at 2 hours was observed in group LC compared with group C, even though this difference was not statistically significant (–47.4% ± 23.3% vs –38.2% ± 25.0%, respectively; P = NS).

Insulin sensitivity was enhanced by L-carnitine supplementation. In group C, plasma insulin levels and

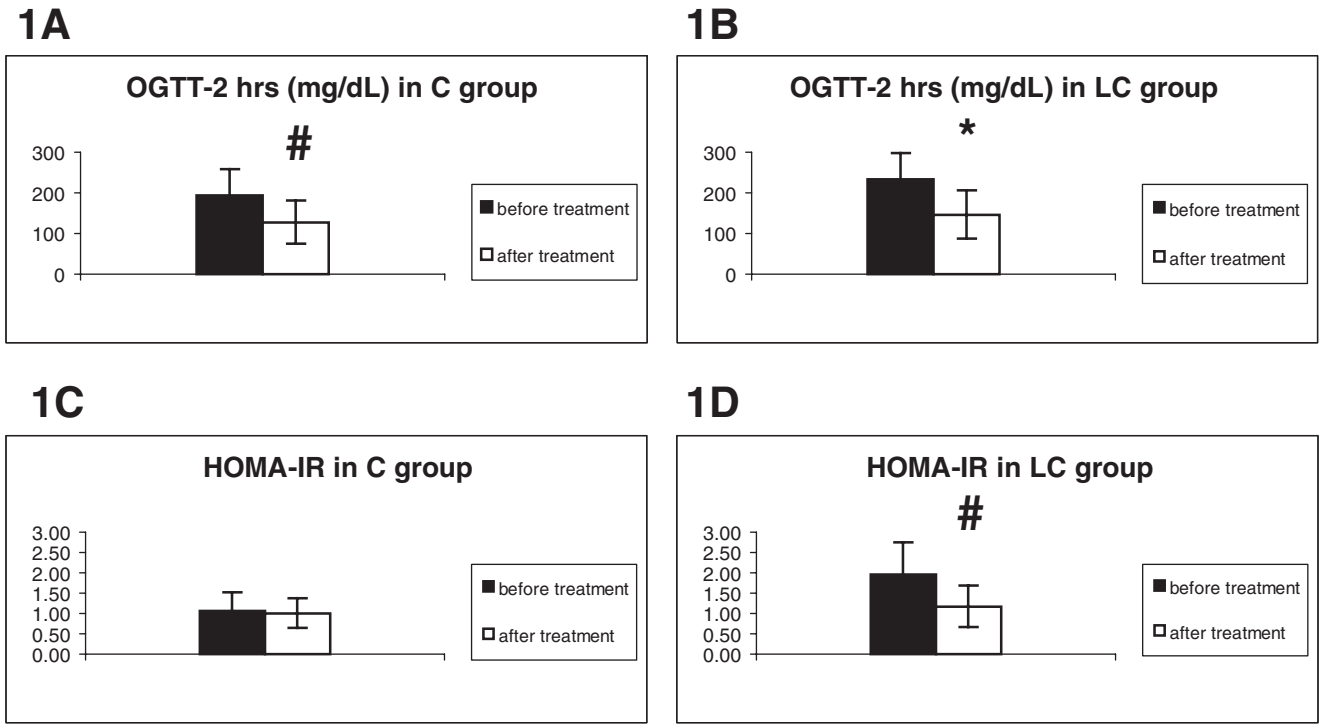

Figure 1. Both groups showed a significant reduction of oral glucose tolerance test (OGTT) 2 hours when compared to baseline value (1A, 1B), but the improvement observed in L-carnitine (LC) patients (*$P < .01$) was more pronounced than in control (C) patients (#$P < .05$). A significant reduction of homeostasis model assessment of insulin resistance (HOMA-IR) was observed in the LC group (1D, #$P < .05$), but it did not change in C group (1C).

HOMA-IR did not change at the end of the study period when compared to baseline values, whereas it significantly decreased in the LC group. Plasma insulin dropped from the baseline concentration of 7.0 ± 2.6 to 4.5 ± 1.7 μU/mL ($P = .04$) at the end of the study. Similarly, HOMA-IR decreased from 1.9 ± 0.7 to 1.1 ± 0.5 ($P = .03$; Figure 1D).

Discussion

Our present data support the role of carnitine in influencing insulin resistance in humans by showing that L-carnitine administration in association with a hypocaloric diet reduces plasma insulin levels and improves insulin resistance in DM-2 and IFG patients. We acknowledge that the small sample size enrolled in this pilot study does not allow definitive conclusions on the role of oral L-carnitine administration in DM-2 and IFG patients to be drawn. However, it is important to note that the 2 groups were not statistically different at baseline, suggesting that the changes observed in the parameters studied were due to the metabolic effects of L-carnitine administration. More important, by analyzing our data in comparison to the existing literature, a new paradigm for the use of L-carnitine supplementation in patients with DM-2 and IFG could be proposed.

The mechanisms by which L-carnitine may enhance glucose metabolism are still to be clearly determined. Insulin resistance appears to be related to mitochondrial dysfunction,[1] which consists, at least in part, of deficient fatty acid oxidation. Inefficient oxidative phosphorylation possibly leads to increased oxidative stress and especially triglyceride accumulation in skeletal muscles, which reduces insulin sensitivity.[13] The biochemical mechanisms responsible for lower fatty acid oxidation involve reduced carnitine palmitoyltransferase (CPT) activity,[13] and exogenous carnitine supplementation may restore the deficit.

Supporting the role of enhanced mitochondrial activity in mediating the metabolic effects of carnitine supplementation, in vitro studies showed that L-carnitine deficit downregulates the mRNA expression of the carnitine acyltransferases, CPT1A and CPT2, and of carnitine acetyltransferase,[14] whereas L-carnitine supplementation completely reverts this downregulation and increases gene expression manifolds.[14] Considering that plasma carnitine concentrations are lower in diabetic patients than in healthy individuals,[2,3] it is likely that the expression and activity of carnitine acyltransferases are reduced, whereas L-carnitine supplementation may contribute to normalizing this metabolic derangement.

The intracellular homeostasis of carnitine is also controlled by different membrane transporters. The membrane potential–driven organic cation transporters (OCTNs),

among which OCTN2 is physiologically the most important, operate on the intestinal absorption and renal reabsorption of L-carnitine and play a major role in tissue distribution. Karlic et al[15] showed that carnitine acyltransferases and OCTN2 are downregulated in healthy elderly patients. However, L-carnitine supplementation does not appear to increase intestinal carnitine absorption.[16] Therefore, it is likely that the metabolic effects of L-carnitine supplementation are mainly due to the induction of the expression of key genes involved in mitochondrial activity, with these effects being minimally influenced by increased carnitine absorption.

Indirect evidence supporting the carnitine-based approach to the treatment of insulin resistance demonstrates that L-carnitine supplementation improves skeletal muscle lipid content and oxidative stress in rats fed a high-fructose diet and ameliorates insulin sensitivity.[17,18] More recently, L-carnitine supplementation has been demonstrated to reduce oxidative stress in diabetic patients,[19] strengthening the role of mitochondrial function in mediating insulin resistance. Furthermore, it should be acknowledged that enhanced mitochondrial function may improve insulin sensitivity beyond its effects on intracellular lipid content. Indeed, muscle contraction improves insulin sensitivity by increasing mitochondrial energy metabolism rather than by lowering the intracellular concentrations of those lipid intermediates presumed to trigger insulin resistance.[20,21]

L-carnitine administration may also be beneficial to glucose metabolism by enhancing the regeneration of the endocrine pancreas by modulating the insulinlike growth factors (IGFs)/and IGF binding proteins. In streptozotocin-induced diabetic rats, liver IGF-1 mRNA expression is reduced but is restored by L-carnitine supplementation.[22] Interestingly, recent experimental data show that IGF-1 regenerates the endocrine pancreas in type 1 diabetes mellitus (DM-1).[23] Whether L-carnitine supplementation may yield metabolic benefits to patients with DM-1 remains to be assessed.

In our pilot study, all patients received a hypocaloric diet. Fasting is known to induce hypothalamic AMP-activated protein kinase (AMPK), a potent modulator of energy homeostasis.[24] Although it is unclear whether 10 days of caloric restriction is enough to induce its expression, it is well known that AMPK triggers a number of molecular events, including increased CPT1 activity and downregulation of fatty acid synthase.[25] However, patients in both groups were calorie restricted, and significant effects on plasma insulin levels and insulin resistance, as assessed by HOMA-IR, were obtained only in patients supplemented with L-carnitine. Moreover, although adipose tissue influences insulin resistance,[26] it is unlikely that a short period of caloric restriction could have significantly reduced fat mass and thus altered patients' hormonal milieu.

Conversely, recent evidence strongly suggests that caloric restriction may be key in obtaining metabolic benefits when L-carnitine is administered. As previously mentioned, the supplementation of L-carnitine to patients with DM-2 or IFG yielded contrasting results. Gonzalez-Ortiz et al[11] recently showed that 4 weeks of L-carnitine supplementation does not improve insulin sensitivity in DM-2 patients. In contrast, our results show that a shorter period of L-carnitine supplementation enhances glucose metabolism in IFG and DM-2 patients. Although it could be postulated that these strikingly different results are related to the different daily doses of L-carnitine (3 g/d in their study[11] vs 4 g/d in our study), the key factor appears to be represented by the prescription of the hypocaloric diet. Indeed, a mild caloric restriction was prescribed to our patients, whereas patients enrolled in Gonzalez-Ortiz et al's study[11] maintained their eating habits. Recent experimental data show that fasting and caloric restriction increase the expression of OCTN2 and carnitine concentrations in the liver and kidney.[27] Therefore, it is conceivable that L-carnitine supplementation at the dosages generally used in clinical practice (2–4 g/d) may not be sufficient to acutely influence carnitine concentrations in tissues, particularly if the administration period is relatively short. Therefore, the expression of key genes of mitochondrial activity may not be affected. However, when L-carnitine supplementation is associated with fasting or caloric restriction or when a peroxisome proliferator-activated receptor alpha (PPAR-α) agonist is concomitantly prescribed,[16] the resulting increased expression of OCTN2 may favor and increase the uptake of carnitine and its rapid accumulation in tissues. Thus the association of L-carnitine supplementation and caloric restriction may allow for carnitine-specific metabolic benefits without requiring a long period of administration.

Considering the limited number of patients enrolled, our results should be considered as preliminary. However, recent data seem to confirm our approach. Supporting our results, Malaguarnera et al[19] showed that L-carnitine supplementation in diabetic patients does not improve fasting glucose levels but significantly ameliorates glycosylated hemoglobin, which is consistent with our data showing a reduction of plasma insulin levels and an improvement of HOMA-IR.

In summary, our results seem to indicate a new paradigm for the use of L-carnitine supplementation in patients with DM-2 and IFG. This paradigm recommends that L-carnitine supplementation coupled with caloric restriction or the prescription of PPAR-α agonists increases the intestinal uptake of carnitine. The combined effects of L-carnitine supplementation and increased absorption may rapidly lead to increased carnitine concentrations in tissues, which results in increased expression of carnitine acyltransferases. Consequently, mitochondrial activity is enhanced and fatty acid oxidation is increased, yielding improved glucose metabolism. Also, reduced hepatic and skeletal muscle lipid

content may further contribute to these clinically relevant metabolic effects.

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